NTP
National Toxicology Program

Breakout Report back

Group 2: Assays and Technologies

(1) Benefits to using mechanistic data

- Human relevance, Efficiency/throughput, Predictive insight that can be applied to other compounds
- Distinguish a particular compound from others in the same class despite similar in vivo endpoints
- By generating large mechanistic datasets, we will be able to identify mechanistic information that is actually well correlated with tumor endpoints
- Represent genetic variability – donor specific individual assays
- By covering all the mechanistic endpoints you could convince regulators that compounds are safe
- Ability to generate large enough datasets that you can overwhelm potential issues with individual datastreams

(1) Challenges to using mechanistic data

- Interpreting mechanistic data in the context of real world exposures
- Codified regulatory issue – individual regulators with different experiences/confidence in different data sources
- Many mechanisms are complex enough that they can only be represented in vivo (e.g. inflammation, immunity, epigenetic)
- Archive and collect data in a way that makes it useful (FAIR)
- Surrogate for in vivo measurement? Human epidemiology?
- Understand cumulative exposure over time and generate mechanistic data based on those conditions
- Balance between privacy/competitive advantage and data sharing

(2) Progression of cancer development

Tipping point to malignancy? Evidence?

- Depends on perspective – everything is based on probability and acceptable risk
- Have to look at tissue-specific signals – what may be malignant in isolation may not be the same in other settings (e.g. breast vs brain)
- Analysis of tipping point is ineffective (has been tried and failed) – it is entirely specific to tumor type and situational influences – moving target

Necessary or sufficient? Individually or in combo?

- Mutations and instability accumulate over time – identify mutational signatures that translate to tipping points
- Indicators of tipping point:
  - genomic instability and de-differentiation
  - Atypical/independent growth
  - EMT
  - Angiogenesis
  - Loss of p53/PTEN activity
  - Metastasis (may be too late)
  - Hyperplasia is insufficient

(3) Technologies and platforms

In vivo

- Look at human cancer hotspots, do nontargeted screening, take biosamples (for further testing once human biomarkers are developed), also look at occupational exposures
- Quantify how in utero exposure to endocrine disrupters would change susceptibility (e.g. transgenic mouse model that has baseline tumor burden that could be increased)
- Transcriptomics in short term animal models – have to be linked to disease endpoints or at least changes in cellular phenotypes (functional or morphological)
- Introduce human cancer cells into ZF – build tumor microenvironment in fish – inject human macrophages and reproduce tumor promotion/suppression
- Develop systems (e.g. imaging) that are better at identifying in situ changes that are indicative of tumors than pathologists – at an earlier time point (to shorten the bioassay)
- Ultra deep sequencing to measure early mutation events

(3) Technologies and platforms

In vitro

- Practical considerations – high availability of “normal” breast tissue from reduction surgeries, compare with genetically susceptible breast tissue from BRCA1+ preventative mastectomies
- In vitro transcriptomics paired with short-term animal studies
- High content screening systems to measure **lack of reversibility** – requires time scale component

(3) Technologies and platforms

In silico

- Mine biological data from genetic mouse models to prioritize chemical-KC perturbations to study
- Computational models are useful to show what is physically impossible, also for hypothesis generation and deciding what type of testing to do
- Opportunity to use “failed” mechanistic data (cytotoxic doses, single time points) to understand how to better design studies, models, and WoE approaches

(4) Building scientific confidence

- Test chemicals for which we know disease output and run transcriptomics to associated dose with endpoints (other cellular measurements as well)
- Parallelogram approach – in vitro animal, in vitro human, in vivo animal
- Define degree of predictivity in the context of animal variability – interspecies predictivity and reproducibility (extrapolate from other tox endpoints where we have multiple studies)
- Validation by external scientific bodies – develop lexicon of studies and applications
- Building confidence depends on application (hazard vs risk based)
- Need good quality epidemiology data and biomarkers to increase the veracity of the findings.
- Need to link findings to the human situation; animal studies are the surrogates. Use of biomarkers measured in human studies

(4) Risk communication

- Computational framework fed by mechanistic data: Tiered Bayesian approach that provide quantitative probabilities – populate such models with well understood chemicals to help convince regulators (learn from failure analysis to identify need for redundant systems, severity of consequences)
- Higher acceptable level of uncertainty because human experiment is already ongoing

(5) What should we be studying?

- Both: look at interaction between multiple carcinogens that act at different sites and non-carcinogens that might be promoting
  - one carcinogen at low dose below threshold + multiple non-carcinogens that interact with the KCs/HMs,
  - same mixture without carcinogen,
  - carcinogen by itself
  - multiple dose levels and multiple time points
- Understand relationship between KCs and susceptibility to cancer – how do each of the KCs modify risk (e.g. time to tumor, dose-response)?
- Pick chemical mixtures with a high public health impact (ground water, air); build model from bottom up?

Consider: Tractability, Interpretability, and Impact of research

(6) How should we be studying joint action?

Disease-centered approach considerations

- Recommended cancer type(s) for study:
- Understand the politics and perception involved in the choice – can better identify quantifiable health impact with specific cancer types, in particular hormonally driven (e.g. breast cancer)

(6) How should we be studying joint action?

Model-based approach considerations

- Recommended animal model(s):
- Sunlight induced melanomas/carcinomas – understand exposures and risk factors – measure thymidine dimers and number of carcinogenic cells – use RHE models and transfer into nude mice – use to develop linkages between underlying genetics and pathology
- Transcriptomics in short-term animal models + in vitro

(6) How should we be studying joint action?

Pathway-driven approach considerations

- Priority pathway combinations:
- Must link KC measurements to self-perpetuating changes in cell proliferation – use transgenic mouse model with baseline tumor burden and test each KC
- Combine 3D culture systems with intermediate animal models and computational modeling